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Influence of dialysate calcium concentration and vitamin D on serum parathyroid hormone during repetitive dialysis

ROGER BOUILLON, RENÉ VERBERCKMOES and PIETER DE MOOR

Laboratory for Experimental Medicine and Clinic of Internal Medicine, Section of Nephrology, Katholieke Universiteit te Leuven, Belgium

Influence of dialysate calcium concentration and vitamin D on serum parathyroid hormone during repetitive dialysis. An acute rise or decrease in parathyroid hormone (PTH) secretion was found in 30 patients, dialyzed with, respectively, low (5 mg/100 ml) or high (7.5 mg/100 ml) calcium concentration. The percentage changes were, respectively, +35% and −47% when a N-terminal antiserum measuring predominantly the glandular PTH was used. Only relatively small changes, respectively, +3% and −17%, were found using a C-terminal antiserum which detects preferentially smaller PTH fragments. Predialysis serum PTH concentration increases significantly with increasing duration of repetitive hemodialysis treatment using an intermediate (6 and 6.4 mg/100 ml) concentration of calcium in the dialysate. No such increase could be found in two other groups of patients treated with high-calcium (7.5 mg/100 ml) dialysis. Moreover, a significant but temporary decrease in predialysis serum PTH concentration occurred two months after a rise in dialysate calcium concentration from 6 to 7.5 mg/100 ml. Treatment with pharmacologic doses of vitamin D3 in selected patients (renal osteodystrophy or children) always resulted in a definite suppression of serum PTH concentration during 14 treatment periods in ten patients. After cessation of vitamin D3 treatment, serum PTH concentration returned to high levels in four out of five patients. These data fail to confirm the long-term involution of secondary hyperparathyroidism using high-calcium dialysis. Vitamin D treatment, however, results in a much more pronounced decrease in serum PTH concentrations, but sustained therapy is necessary.

Influence de la concentration du calcium dans le bain de dialyse et de la vitamine D sur la concentration sérique d'hormone parathyroïdienne au cours de l'hémodialyse itérative. Une augmentation ou une diminution aiguës de la sécrétion d'hormone parathyroïdienne (PTH) a été observée chez 30 malades dialysés, respectivement, avec des concentrations de calcium faible (5 mg/100 ml) ou élevée (7,5 mg/100 ml). Les pourcentages de modification ont été respectivement de +35% et −47% quand un antisérum N terminal, qui mesure surtout la PTH glandulaire, a été utilisé. Des modifications minimes, respectivement, 3% et −17%, ont été mises en évidence par un antisérum C terminal qui détecte préférentiellement des fragments plus petits de la PTH. La PTH sérique avant la dialyse augmente significativement en fonction de la durée du traitement par l'hémodialyse quand une concentration de calcium intermédiaire (6 et 6,4 mg/100 ml) est utilisée. Cette augmentation n'est pas constatée chez les malades de deux autres groupes traités avec des concentrations de calcium élevées (7,5 mg/100 ml). De surcroît, une diminution significative mais éphémère de la concentration sérique pré-dialytique de PTH est survenue deux mois après l'augmentation de la concentration du calcium du bain de 6 à 7,5 mg/100 ml. Le traitement par des doses pharmacologiques de vitamine D3 chez des malades sélectionnés (ostéodystrophie rénale, enfants) a toujours pour conséquence une suppression nette de l'augmentation de la PTH sérique, cela au cours de 14 traitements chez dix malades. Après l'arrêt de la vitamine D3 chez cinq malades la PTH sérique est revenue à des valeurs élevées chez quatre d'entre eux. Ces résultats ne confirment pas la réduction à long terme de l'hyperparathyroïdisme secondaire par l'utilisation d'un bain de dialyse riche en calcium. Le traitement par la vitamine D, cependant, a pour conséquence une diminution beaucoup plus importante de la PTH sérique mais un traitement ininterrompu est nécessaire.

Renal insufficiency, either acute or chronic, is associated with secondary hyperparathyroidism. This has been confirmed by morphological findings in the parathyroid glands [1], by the presence of specific bone lesions [2] and also by direct measurement of serum or plasma parathyroid hormone concentration [3–5]. These parathyroid hormone (PTH) measurements, however, must be interpreted carefully because of the altered distribution of different PTH components in the presence of renal insufficiency. Indeed, the intact glandular hormone (mol wt ± 9,500) retains its normal short half-life (± 20 min), but the main breakdown product, the biologically inactive "peripheral" parathyroid hormone (mol wt ± 6000 to 7000) accumulates in serum since its half-life increased markedly (> 24 hr, [6]).

The pathogenetic mechanisms responsible for this secondary hyperparathyroidism are essentially known: phosphate retention [7], abnormal metabolism of vitamin D [8, 9] and skeletal resistance to the action of parathyroid hormone [10]. The exact interrelationship and the sequential importance of these factors, how-

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ever, are still disputed [11]. All factors mentioned, however, tend to decrease ionized serum calcium concentration, which is known to be the best stimulus for parathyroid hormone secretion.

Since secondary hyperparathyroidism, although adaptive in origin, can have deleterious effects on bone, especially in the case of prolonged survival due to successful repetitive hemodialysis, different kinds of therapy and prevention have been proposed: diminution of the phosphorus intake or of its gastrointestinal absorption [12], calcium supplementation orally [13], higher calcium concentration in the dialysate [14] and administration of vitamin D or its analogues [15].

For better understanding of these therapeutic possibilities, we have studied the short- and long-term influence of dialysate calcium concentration and vitamin D3 treatment on parathyroid hormone secretion in patients receiving repetitive long-term hemodialysis (Table 1).

Methods

Patients. Patients with end-stage renal insufficiency necessitating chronic hemodialysis were studied. They all received aluminum hydroxide orally in a nearly constant dose (2.5 to 6 g/day) during the observation period.

In *group A* serum for measurements of calcium, phosphorus and alkaline phosphatase was taken before and at the end of their usual eight-hour dialysis. Parathyroid hormone was also measured using two different antibovine parathyroid antisera (BW 211/32 and A-VI-2, see below). In a first experiment (4/15/1973) the usual dialysate calcium concentration of 7.5 mg/100 ml was used but in the second (5/15/1973) the dialysate calcium was only 5 mg/100 ml.

In *group B* the dialysate calcium concentration was 6 mg/100 ml until 11/2/1972 and 7.5 mg/100 ml afterwards. All other dialysate constituents remained unchanged (sodium, 137 mEq/liter; potassium, 1.0 mEq/liter; acetate, 35 mEq/liter; magnesium, 1.0 mEq/liter). Dialysis was performed during eight hours twice-weekly with a twin-coil kidney. Serum for measurement of parathyroid hormone was obtained before the start of the dialysis three times before and eight times after the rise in dialysis calcium concentration. The total observation time ranged from 4 to 15 months after the rise in dialysate calcium. Predialysis serum calcium and phosphorus concentrations and alkaline phosphatase activity were measured twice monthly and the mean value for each patient during the month preceding the sampling for serum PTH was calculated. Two patients died during the last month of the observation: one from overhydration and the other from a cerebrovascular accident. One other patient was transferred to another dialysis center for social reasons (in September, 1973).

Group C was started on regular hemodialysis only

Table 1. Diagram of the different study protocols and their aims in the study of parathyroid hormone secretion

Group	Patients *N* (M/F)	Age of patients (range) *yr*	Duration (*months*) of previous hemodialysis twice weekly, 8 hr (range)	Dialysate calcium	Selection of patients	Anti-PTH antiserum	Aims of the study: Influence on PTH secretion
A	30 (21/9)	38 (16–67)	73 (1–321)	7.5 5	No	N-terminal and C-terminal	Acute study: Effects of different dialysate calcium concentrations
B	10 (6/4)	31 (20–44)	23 (2–75)	6 7.5	Exclusion of patients with criteria for group E	N-terminal	Chronic study: a) idem as for group; b) prospective study of a rise in dialysate calcium concentration from 6 to 7.5 mg/100 ml
C	8 (5/3)	46 (22–67)	2 (0.5–4.5)	7.5	Exclusion of patients with criteria for group E	N-terminal	Chronic study: prospective study of the influence of a dialysate calcium concentration of 7.5 mg/100 ml
D	26 (14/12)	38 (6–54)	9 (1–44)	6.4	No	N-terminal	Chronic study: cross-sectional study of the influence of duration of chronic hemodialysis
E	10 (5/5)	28 (13–49)	13 (0–42)	7.5	Severe radiological signs of renal osteodystrophy or young age (<18)	N-terminal	Chronic study: prospective study of the influence of vitamin D therapy

after 11/2/1972, each time using a dialysate calcium concentration of 7.5 mg/100 ml. Predialysis serum PTH measurements were performed six to eight times in each of these patients during a mean total period of nine months (range: seven to ten months).

Group D was treated with regular hemodialysis at another hospital (University Hospital, Ghent, Service: Prof. R. Ringoir) with a dialysate calcium concentration of 6.4 mg/100 ml. Serum for PTH measurement was taken only once before the start of dialysis.

Group E was treated with pharmacologic doses of vitamin D3 because of typical signs of osteitis fibrosa or because of their young age. The clinical history of some of these patients has been discussed previously [16]. Serum for parathyroid hormone measurement was taken at regular intervals before, during and after the end of vitamin D treatment (which was discontinued when the serum calcium concentration increased to >11 mg/100 ml or radiographic signs of renal osteodystrophy had disappeared). Further treatment consisted of regular hemodialysis as for group A patients and oral administration of aluminum hydroxide in constant dosage.

Methods. Serum calcium and phosphorus concentrations and alkaline phosphatase activity were measured with the aid of an autoanalyzer. Normal adult values for serum calcium range from 8.9 to 10.6 mg/100 ml, 2.5 to 4.25 mg/100 ml for serum phosphorus and 40 to 140 IU/ml for serum alkaline phosphatase. Serum parathroid hormone was measured with a radioimmunoassay method, previously described in detail [17, 18]. The final serum concentration was 10 to 20% in a total incubation volume of 0.5 ml. Phase separation was performed with dextran-coated charcoal (20 mg of charcoal, Norit A; 3 mg of Dextran 70) after non-equilibrium incubation for seven days at 4°C. Purified bovine PTH (gift of Dr. G. Aurbach) was used as an internal standard since previous experiments indicated a parallel inhibition with serum of hyperparathyroid patients diluted serially with serum of hypoparathyroid patients. Antiserum BW 211/32, which mainly reacts with the glandular form of the hormone and also cross-reacts with the synthetic 1–34 bPTH fragment, was used in all studies. For the experiments on patients of group A and for the gel filtration studies another antiserum, A-VI-2, was also used. This antiserum does not cross-react with the biologically active N-terminal fragment and recognizes mainly a parathyroid hormone fragment with a mol wt of about 7,000. Normal values for serum parathyroid hormone are 210 pg of bovine PTH/ml for antiserum BW 211/32 and <400 pg of bPTH/ml for A-VI-2. The between-assay variation coefficient was 10.8% for a sample with 1513 pg/ml and 7.7% for a sample with 282 pg/ml, both measured with antiserum BW 211/32. For antiserum A-VI-2 this variation coefficient was 7.8% for a sample with 1954 pg/ml and 8.7% for a sample with 1193 pg/ml.

Gel filtration on Bio Gel P-10 (Bio-Rad) was performed on columns of 16×700 mm at 4°C using a slightly acidic buffer (NaCl, 0.14 M; bovine serum albumin, 0.25%; pH 3 with HCl). Fractions of 2 ml were collected at a flow rate of 12 ml/hour using a peristaltic pump and an automatic fraction collector. The elution position of glandular human parathyroid hormone and ^{125}iodide was identified by a previous run on the same column in identical circumstances. One-tenth of each fraction (0.2 ml) was assayed for parathyroid hormone immunoreactivity with both antisera (BW 211/32 and A-VI-2). The results were expressed as percent inhibition of the binding of tracer bPTH to its antiserum in the absence of added cold hormone. This expression was chosen because it is probable that not all fractions diluted out linearly to the glandular bPTH standard.

Results

A. Short-term effects of dialysate calcium on serum parathyroid hormone levels. a. Dialysate calcium concentration of 7.5 mg/100 ml. Mean serum calcium concentration in 30 patients attending the dialysis center (group A) increased from a predialysis value of 9.38 ± 0.19 (mean ± SEM) to 11.19 ± 0.2 mg/100 ml at the end of hemodialysis ($P<0.001$). Serum phosphorus concentration decreased and serum alkaline phosphatase activity increased significantly (Fig. 1). The calcium × phosphorus product decreased from 43.3 ± 2 to 37.8 ± 2 ($P<0.05$).

Serum parathyroid hormone, measured with antiserum BW 211/32, decreased from 666 ± 165 to 350 ± 101 pg bPTH/ml ($P<0.01$). This is a mean percentage decrease of 47.5%. When, in the same samples, parathyroid hormone was assayed with antiserum A-VI-2, a decrease from 3019 ± 407 to 2495 ± 415 pg of bPTH/ml occurred ($P<0.001$). This is a percentage decrease of only 17.4% (Fig. 1).

b. Dialysate calcium of 5 mg/100 ml. When the 30 patients of group A were dialyzed with a dialysate calcium of only 5 mg/100 ml, the mean predialysis serum calcium concentration (9.36 ± 0.13 mg/100 ml) decreased to 8.93 ± 0.12 at the end of the dialysis ($P<0.001$). Mean serum phosphorus concentration also decreased and serum alkaline phosphatase activity again increased significantly (Fig. 1). The calcium × phosphorus product decreased from 42.7 ± 2.5 to 31.5 ± 1.4 ($P<0.001$). Serum parathyroid hormone measured with antiserum BW 211/32 increased from a

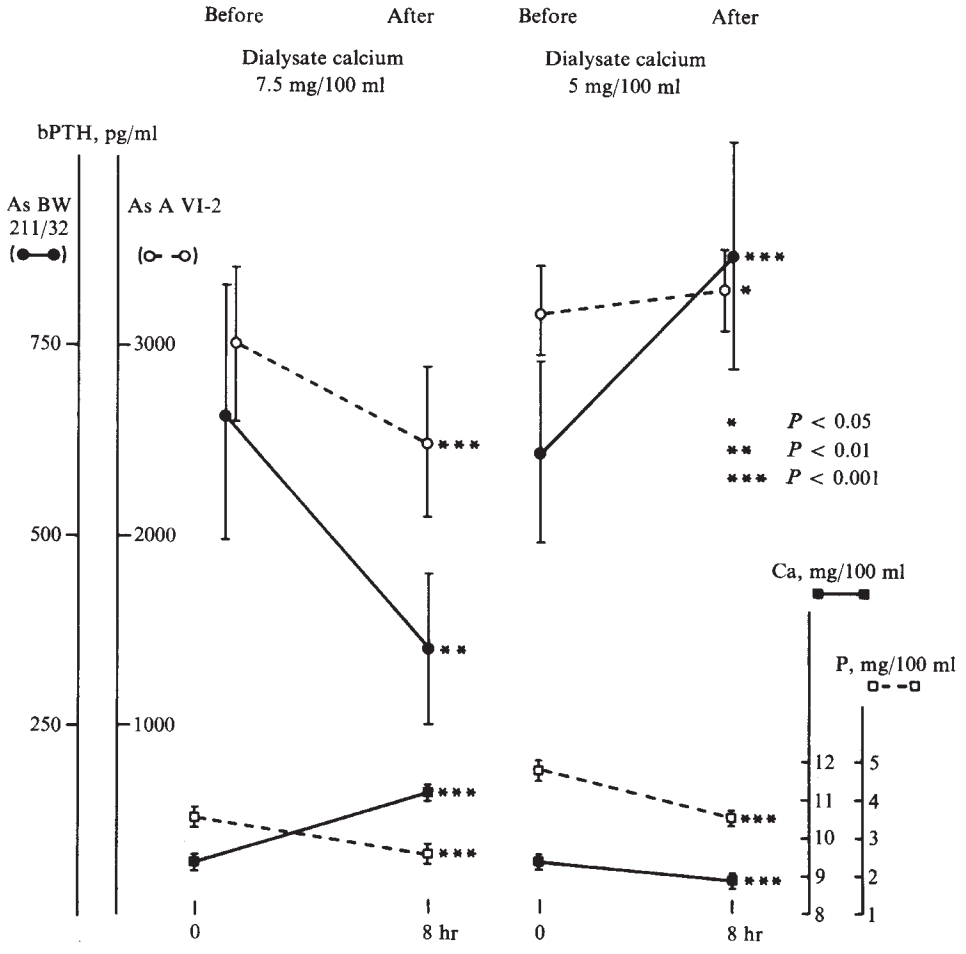

Fig. 1. *Mean (± SEM) pre- and postdialysis serum concentrations of calcium, phosphorus, alkaline phosphatase and parathyroid hormone, measured with two different antisera, in 30 patients (group A) during a single dialysis with low (5 mg/100 ml) or high (7.5 mg/100 ml) calcium concentration.*

predialysis value of 606 ± 119 to 820 ± 152 pg of bPTH/ml ($P < 0.001$) at the end of dialysis; measured with antiserum A-VI-2 a small increase from 3186 ± 242 to 3294 ± 235 pg of bPTH/ml occurred ($P < 0.05$). End-dialysis values were thus respectively 135.3% and 103.4% of the predialysis value for the two antisera used (Fig. 1).

c. Gel filtration of serum before and after dialysis. Two serum samples (2 ml) from the same patient, one taken before and one at the end of hemodialysis with a dialysate calcium concentration of 7.5 mg/100 ml were analyzed by gel filtration on a Bio-Gel P-10 column. Predialysis serum parathyroid hormone eluted mainly in the same position as a glandular bovine and human PTH extract when antiserum BW 211/32 was used, while antiserum A-VI-2 detected much more immunoreactivity in the lower molecular regions. After dialysis a marked decrease in immunoreactivity as measured with BW 211/32 occurred (from 2420 to 880 pg of bPTH/ml), corresponding to the diminished area of the "glandular" PTH fraction measured with this antiserum in the gel filtration fractions (Fig. 2). However, a much smaller decrease in parathyroid hormone immunoreactivity was found when antiserum A-VI-2 was used as well in the same whole serum (a decrease from 6390 to 4960) as in the same gel filtration fractions (Fig. 2).

B. Long-term influence of dialysate calcium on parathyroid hormone secretion. a. The influence of the duration of previous therapy with regular hemodialysis using a dialysate calcium of 6 mg/100 ml can be appreciated by a positive correlation coefficient ($r = 0.76$, $N = 10$, $P < 0.02$) between duration of previous therapy (90 ± 30 weeks, mean ± SEM) and predialysis serum parathyroid hormone (1069 ± 258 pg of bPTH/ml) in the ten patients of group B (Fig. 3).

In another group of 26 patients (group D) treated at another hospital (University Hospital, Ghent) with a dialysate calcium of 6.4 mg/100 ml, a similar positive correlation coefficient ($r = 0.50$, $N = 26$, $P < 0.01$) could be found between the duration of previous hemodialysis therapy (35 ± 8 wks) and predialysis serum parathyroid hormone levels (480 ± 94 pg of bPTH/ml) (Fig. 3).

b. Long-term influence of a rise in dialysate calcium

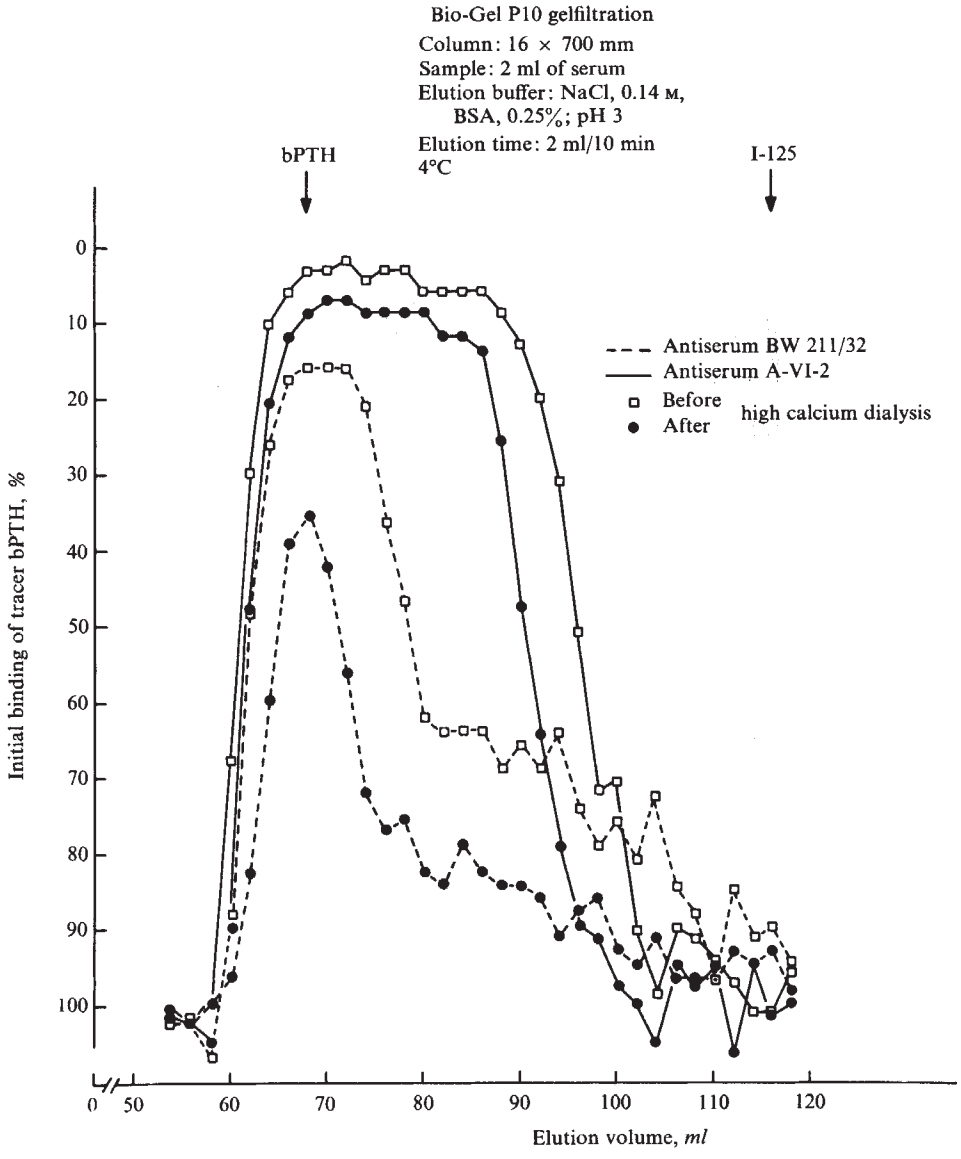

Fig. 2. *Gel filtration on Bio Gel P-10 of 2 ml of serum taken before* (□) *or after the end* (●) *of hemodialysis with high (7.5 mg/100 ml) calcium concentration in patient 16.* Each fraction (2 ml) was assayed with two different antiparathyroid hormone antisera: BW 211/32 (- - - - -) and A-VI-2 (———).

concentration (*patients of group B*). Mean predialysis serum calcium concentration measured during three periods of one month in the ten patients of group B were, respectively, 9.26 ± 0.16 (mean ± SEM), 8.97 ± 0.17 and 9.26 ± 0.16, when the dialysate calcium concentration was only 6 mg/100 ml. After the rise in dialysate calcium concentration to 7.5 mg/100 ml, the predialysis serum calcium concentration increased in all patients to a mean of 10.17 ± 0.22 in January, 1973 and 10.20 ± 0.18 in February, 1973 (paired *t* test compared to October, 1972: $P < 0.01$). During further follow-up, however, predialysis serum calcium levels decreased again (Fig. 4).

Predialysis serum phosphorus concentration decreased from 4.10 ± 0.33 and 4.84 ± 0.44 during the low dialysate calcium period to 3.45 ± 0.21 and 3.40 ± 0.27 in the January and February period, respectively, ($P < 0.025$).

Mean predialysis serum alkaline phosphatase was higher than normal during the low dialysate calcium period (241 ± 49 to 261 ± 55 IU/ml). A significant decrease was not found until five months after the rise in dialysate calcium concentration.

Predialysis serum parathyroid hormone levels, measured three times during the low dialysate calcium period did not change significantly. However, two months after the rise in dialysate calcium concentration to 7.5 mg/100 ml, a decrease in predialysis serum PTH levels was found (645 ± 173 pg of bPTH/ml). This value was significantly different from the previous October, 1972 value of 1069 ± 258 when calculated with a paired *t* test ($P < 0.05$). Because some doubt

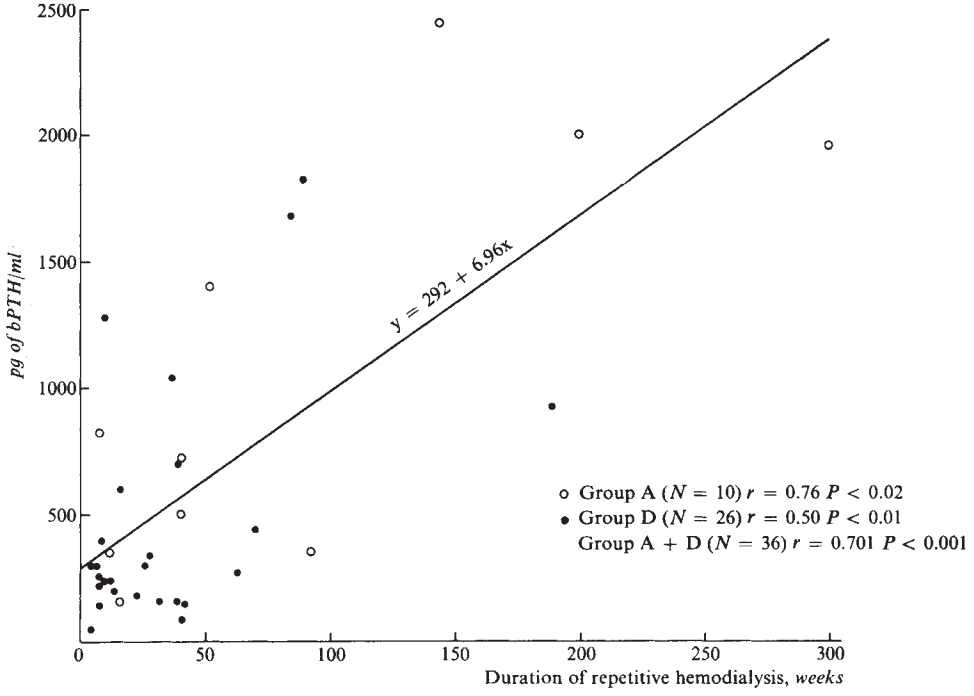

Fig. 3. *Correlation between total duration of previous treatment with repetitive hemodialysis, using an intermediate (6 to 6.4 mg/100 ml) dialysate calcium concentration, and predialysis serum parathyroid hormone concentration (antiserum, BW 211/32).*

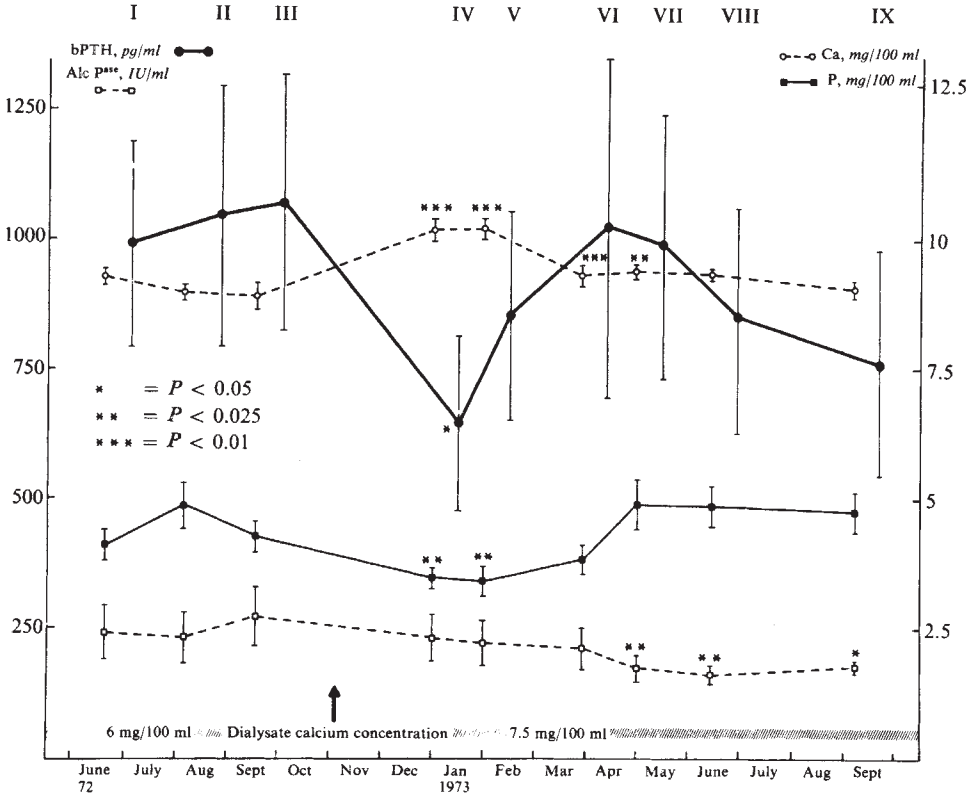

Fig. 4. *Mean (± SEM) predialysis serum concentration of calcium, phosphorus, alkaline phosphatase and parathyroid hormone (antiserum, BW 211/32) in ten patients (group B) dialyzed with intermediate (6 mg/100 ml) and high (7.5 mg/100 ml) calcium concentration.*

about the normal distribution of these values could exist the same data were analyzed statistically using a Wilcoxon rank sum test for paired data ($2\alpha = 0.05$) and a paired Student's t test after logarithmic transformation ($P < 0.02$). During the subsequent months, however, serum PTH levels increased again progressively and were no longer different from the levels obtained during the period of low calcium dialysate (Fig. 4).

c. Influence of prolonged treatment with high calcium dialysate on serum parathyroid hormone (group C patients). Mean predialysis serum parathyroid hormone levels obtained in the first and second month of observation were, respectively, 335 ± 69 and 404 ± 61 pg of bPTH/ml in the eight patients of group C. After treatment with long-term repetitive hemodialysis using a high calcium dialysate (7.5 mg/100 ml) during a mean period of seven and nine months, the predialysis serum parathyroid hormone concentration had not changed significantly (Table 2).

C. *Influence of pharmacologic doses of vitamin D3 on parathyroid function (group E).* The high predialysis serum parathyroid hormone levels before (1410 ± 224

Table 2. Individual results of predialysis serum parathyroid hormone (PTH) measurements (N-terminal antiserum, BW 211/32) in eight patients (group C) who always have been treated with high-calcium (7.5 mg/100 ml) dialysis

Patient No.	Sex	Age *yr*	Observation period				Duration of follow-up I-IV *months*
			I	II	III	IV	
1	M	61	100	300	170	340	10
2	M	34	460	620	450	560	10
3	M	53	650	490	380	680	10
4	F	22	260	540	320	190	10
5	M	40	360	380	190	250	10
6	F	54	440	380	380	440	9
7	F	37	360	460	740	440	7
8	M	67	50	60	480	230	7
Mean PTH, *pg/ml*			335	404	389	391	
SEM			69	61	64	61	
Paired t test				NS	NS	NS	

Table 3. Age, sex, duration of previous hemodialysis and duration and dosage of vitamin

Patient No.	Age *yr*	Sex	Duration of previous dialyses *weeks*	Duration of treatment *weeks*	Dosage of D3/month *mg*	Before			During		
						PTH *pg bPTH/ml*	Ca *mg/100 ml*	AP *IU/ml*	PTH *pg bPTH/ml*	Ca *mg/100 ml*	AP *IU/ml*
9[b]	49	F	6	30	120	2500	9.32	787	1520	9.56	646
10	16	M	13	26	60	2360	9.90	355	612	10.00	300
11	17	M	62	26	60	535	10.68	90	515	11.18	106
12	15	F	2	24	30	1100	9.50	199	440	10.10	141
13	13	M	15	20	60	2120	8.83	280	—	—	—
14[b]	30	M	55	16	180	481	9.47	742	—	—	—
15[b]	44	F	24	2	120	805	10.75	115	—	—	—
16[b] I	30	F	11	28	120	650	9.67	612	780	9.16	174
II	(30)	(F)	76	13	60	350	8.60	101	50	11.60	82
17[b] I	42	F	1	6	120	2520	8.80	935	—	—	—
II	(42)	(F)	14	21	60	480	11.70	1027	295	11.12	648
18[b] I	26	M	130	13	120	2380	8.83	831	920	9.97	450
II	(26)	(M)	148	6	120	940	10.30	217	—	—	—
III	(26)	(M)	168	26	15	2500	9.90	108	870	9.44	147
Mean	28.2		51.8	18.35	89	1410	9.73	485	667	10.24	299
SEM or range	13–49	5M/5F	2–168	2–30	15–180	244 (14)	0.24	96	142 (9)	0.29	121
Paired t test					t	—	—	—	3.16		2.49
Compared with values "before"					P				< 0.02	NS	< 0.05

[a] With individual and mean (±SEM) values of serum calcium, alkaline phosphatase (AP) and parathyroid hormone.
[b] Patients with severe radiological signs of renal asteodystrophy.

pg bPTH/ml) the start of the vitamin D3 treatment decreased significantly during (667 ± 142) and at the end (267 ± 35) of the treatment period (Table 3). Serum calcium was significantly increased in all patients at the end of therapy with mean values slightly above normal (11.23 ± 0.22 mg/100 ml). Serum alkaline phosphatase decreased dramatically to nearly normal values at the end and after arrest of treatment. Serum phosphorus did not change significantly during the whole treatment period. In four out of five patients serum PTH increased again when the serum calcium concentration returned to normal values more than one month after the arrest of vitamin D treatment.

Discussion

Influence of short-term changes in serum calcium on parathyroid hormone secretion. The difference in calcium concentration between the dialysate fluid and the plasma ultrafiltrate is the most important factor regulating the external calcium balance during hemodialysis [19, 20]. In previous studies [21, 22] we found a decrease in the ionized serum calcium concentration during hemodialysis with a dialysate calcium concentration of 5 mg/100 ml, and an increase when a dialysate calcium of >6 mg/100 ml was used. Only the total serum calcium concentration was measured in the present study, but the same significant change in postdialysis serum calcium concentration was found with dialysate calcium concentrations of 5 and 7.5 mg/100 ml, respectively. Serum PTH increased during low-calcium dialysis and decreased during high-calcium dialysis. The (percentage) variations, however, are much more important when PTH is measured with an N-terminal antiserum as compared to those measured with a C-terminal antiserum. This difference in results can be explained by the antigenic characteristics of the two antisera. Because of the short half-life of the intact glandular hormone, even in case of renal insufficiency [23, 24] the values obtained with the N-terminal antiserum reflect the acute response of the parathyroid glands to changes in serum calcium concentration. The decrease of new glandular hormone secretion, however, does not have an immediate effect on the serum concentration of the inactive fragments (measured by antiserum A-VI-2) because of their long half-life in circulation [25]. Possible interference by elimination of small parathyroid hormone fragments through the dialysis membrane can be excluded by the absence of significant amounts of immunoreactivity in the plasma ultrafiltrate, obtained with the membrane used during hemodialysis.

The problem of the autonomy of the parathyroid glands in patients with renal insufficiency has been studied previously during calcium infusion studies by Reiss, Canterbury and Kantor [26], Massry et al [27] and Genuth et al [28] who found parathyroid hormone to decrease in most patients. O'Riordan et al [24], however, could not find such a decrease. Berson and Yalow [29] and Goldsmith et al [23] found differences in parathyroid hormone suppressibility according to the antisera used. Antisera C-329 [29] and CH-14-M [23], measuring mainly the intact hormone, as our antiserum BW 211/32 [18] detected acute decreases in serum parathyroid hormone after important rises in serum calcium concentration. Antiserum 273 [29] and GP-1-M [23] like our antiserum A-VI-2 could only detect minimal and slow decreases. Dialysis with extremely high (11.5 mg/100 ml) or low (3.5 mg/100 ml) calcium concentration could influence serum parathyroid hormone in the same manner in four patients [30] as we found with less extreme variation in dialysate calcium concentrations.

Although the hypersecretion of parathyroid hormone can be suppressed by increasing the serum calcium concentration, normal values were not attained in our patients at the end of the high-calcium dialysis despite a mean supranormal end-dialysis serum calcium of 11.18 mg/100 ml. This resembles the situation in many cases of "primary" hyperparathyroidism where partial suppressibility seems to be more frequent than real autonomy [18]. A possible explanation could

D3 treatment in ten patients (group E)[a]

At the end			One month or more after cessation of treatment		
PTH *pg bPTH/ml*	Ca *mg/100 ml*	AP *IU/ml*	PTH *pg bPTH/ml*	Ca *mg/100 ml*	AP *IU/ml*
460	10.48	470	—	—	—
280	12.80	189	240	10.90	110
250	10.63	115	—	—	—
290	10.30	99	—	—	—
315	11.59	156	—	—	—
370	12.10	331	—	—	—
420	12.16	113	—	—	—
356	11.30	162	1685	10.41	195
60	11.96	93	350	8.80	82
400	11.27	1200	—	—	—
180	10.76	225	—	—	—
210	10.62	209	940	10.30	217
50	11.20	109	1380	10.00	105
100	10.06	113	—	—	—
267	11.23	261	919	10.08	142
35	0.22	83	281	0.35	133
14			5		
4.76	4.47	2.78	—	—	—
<0.001	<0.001	<0.02	NS	NS	NS

be the phenomenon that excess amounts of parathyroid tissue (as can be obtained by isologous parathyroid transplantation [31]) need a higher than usual calcium concentration before total arrest of secretion occurs.

Long-term influence of dialysate calcium on parathyroid hormone secretion. In two independent groups of patients (group B and D) a positive correlation was found between the duration of previous treatment with repetitive hemodialysis and serum parathyroid hormone. This positive correlation indicates that the pathogenic mechanisms of secondary hyperparathyroidism still operate during this kind of low-calcium dialysis. A similar positive correlation was found previously by Johnson et al [32] who used a calcium dialysate concentration of only 2.6 mEq/liter, but Fournier et al [33] were unable to find such a correlation. It is likely that other factors can also influence PTH secretion although we could not find any other significant correlation (age, serum creatinine, phosphorus or calcium) with the PTH level. This progression in secondary hyperparathyroidism is in agreement with previous clinical [34, 35], radiological [27, 36] and histological [37, 38] observations indicating that prolonged treatment with repetitive hemodialysis using low or intermediate calcium concentration (< 6.5 mg/100 ml) in the dialysis fluid aggravates bone lesions.

A high dialysate calcium concentration has recently been advocated for prevention or cure of secondary [14, 39] and even "tertiary" [40] hyperparathyroidism. Indeed, when dialysate calcium and predialysis serum phosphorus concentrations were manipulated [33], the lowest predialysis serum concentration of parathyroid hormone was found when the serum phosphorus concentration was lowered by oral administration of aluminum hydroxide together with the use of what was called "high (> 6 mg/100 ml) calcium dialysis." However, during these experiments no definitely high dialysate calcium was used, the observation periods were short and both dialysate calcium and phosphorus absorption were changed together. Previous measurements of serum parathyroid hormone after short-term high-calcium dialysis indicated a good suppression with 8 mg/100 ml [14, 39] but not with 7 mg/100 ml [32].

Our data on the ten patients of group B indicate that a rise in dialysate calcium from 6 to 7.5 mg/100 ml results in an increase in predialysis serum calcium and a reciprocal decrease in serum parathyroid hormone (period IV; Fig. 4). However, after several months of continuous high-calcium dialysis, a progressive return of serum calcium, serum phosphorus and serum PTH to the previous concentrations occurred despite the fact that all therapy remained the same. This does not mean that a high dialysate calcium was absolutely ineffective because it prevented the prior continuous rise in serum PTH as the duration of repetitive dialysis increased. The same stabilization in serum parathyroid hormone levels was indeed found in a group of patients (group B) who were always treated with high (7.5 mg/100 ml) calcium dialysis (Table 2).

One other group has repeatedly argued that it observes a progressive decrease in serum PTH using high-calcium dialysis [14, 39, 41]. Numerous differences, however, exist between the two study protocols: their dialysis frequency was higher (three times vs. two times a week), their blood sampling was performed the day after dialysis (vs. our predialysis sampling) and their anti-PTH antiserum was C-terminal (vs. our N-terminal one), their patients were new ones and ours were already treated with repetitive hemodialysis before this study. The difference in sampling time is probably not very important but this could be the case for the antisera difference if the parathyroid glands can secrete independently the intact and the inactive fragment. Insufficient data, however, are presently available on this possibility [25]. Two major protocol differences, however, seem to be important: first a higher frequency of dialysis (Mayo Clinic) could have resulted in a more positive calcium balance. This total calcium transfer, a kind of product of the dialysate calcium concentration and duration of dialysis, could be the determinant factor in the PTH secretion. The second important difference is one of patient selection: our patients were already treated with an intermediate calcium dialysis resulting in a nearly normalization of the serum calcium and phosphorus concentrations, which was apparently not the case for the Mayo Clinic patients, who were newly dialyzed patients. Comparing the PTH levels during a period of intermediate calcium (6 mg/100 ml) dialysis and subsequently during high-calcium (7.5 mg/100 ml) dialysis is probably a better indication of the effect of the rise in dialysate calcium concentration per se.

Influence of vitamin D treatment on parathyroid hormone secretion. Although the presence of osteitis fibrosa is usually associated with excessive parathyroid hormone secretion and not with vitamin D deficiency, improvement of this bone lesion during chronic renal failure has been achieved by vitamin D treatment, using either dihydrotachysterol [42] or vitamin D3 [43, 16]. The influence of this treatment on PTH secretion, however, has not been studied previously. During prolonged treatment with pharmacologic doses of cholecalciferol, an important suppression of PTH secretion was found in all our patients. With very few exceptions, a simultaneous increase in serum calcium and a decrease in serum parathyroid hormone and

serum alkaline phosphatase occurred. At the end of treatment, when slight hypercalcemia existed in eight patients, inappropriately high parathyroid hormone levels still existed in six patients. When the vitamin D treatment was arrested, PTH levels increased rapidly again in four out of five patients studied. These two phenomena suggest that in most cases parathyroid hyperfunction is only temporarily suppressed by a higher than normal serum calcium concentration, and that sustained treatment is thus unnecessary. From individual roentgenological and histological bone analysis in our patients, we found [16] that partial healing of the osteitis fibrosa could already occur before the decrease in serum PTH. This suggests that vitamin D treatment together with high-calcium (7.5 mg/100 ml) dialysis first restores a bone calcium deficit and results afterwards in a progressive rise in serum calcium which finally suppresses parathyroid hormone secretion.

Therapeutic considerations. The evolution of serum parathyroid hormone concentrations in patients with renal insufficiency can be important since secondary hyperparathyroidism in this disease can be considered to be an index for the primary disturbance of calcium metabolism [7]. From our long-term studies we can conclude that secondary hyperparathyroidism, once fully developed, is very difficult to cure. The use of a high calcium concentration (7.5 mg/100 ml) in the dialysate fluid can, in the long run, only arrest the progression of the secondary hyperparathyroidism, which normally occurs using intermediate (6 to 6.4 mg/100 ml) dialysate calcium concentrations. Prolonged treatment with vitamin D3 can effectively suppress parathyroid hormone hypersecretion, at least in selected patients, but continuous treatment, with progressively lower doses, is necessary to keep the parathyroid glands suppressed. This is not unexpected since persistent hyperparathyroidism has been demonstrated [44] even more than three years after restoration of normal vitamin D metabolism [45] by successful renal transplantation, thus indicating that involution of hyperplastic parathyroid glands is usually a very slow process. Of course, whether this effective PTH suppression occurred by a direct effect of vitamin D or by its influence on the serum calcium concentration cannot be derived from these studies *in vivo*.

Side-effects, especially metastatic calcification, have previously been reported both during the use of high-calcium dialysis [46] and vitamin D treatment [47]. However, with regular monitoring of the serum electrolyte concentrations (with special attention to continuous normalization of the serum phosphorus concentration), we have been able to see even disappearance of preexisting vascular calcification in patients on long-term repetitive hemodialysis treated with pharmalogic doses of vitamin D3 [48]. Nevertheless, the present doses of vitamin D3 would probably be much too high for patients without manifest renal osteodystrophy.

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Reprint requests to Dr. Roger Bouillon, Laboratorium voor Experimentale Geneeskunde, Kliniek voor Interne Geneeskunde, Katholieke Universiteit te Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium.

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